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COMPARATIVE STUDY OF E-STRIP AND VITEK 2 FOR VANCOMYCIN MIC FOR STAPHYLOCOCCUS AUREUS AT TERTIARY CARE CENTER

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ABSTRACT **PURPOSE:** Comparative Study done for Vancomycin susceptibility in Methicillin Resistant Staphylococcus aureus (MRSA) in two methods Vitek-2 and E-strip test.

MATERIAL AND METHOD: Vancomycin susceptibility testing was performed on these Methicillin Resistant Staphylococcus aureus (MRSA) isolates by two methods viz. Vitek 2 & E- strip Test.

RESULT: A total of 10680 various specimens were received and processed in laboratory. 210 samples were S. aureus amongst which 76 were MRSAs. Pus was the predominant sample followed by endotracheal secretions, blood, and sputum. All the strains of MRSA found susceptible to vancomycin (MIC≤2µg/ ml) by both the methods. Maximum specimens by both the methods had MICs of 1.0 µg/ ml.

CONCLUSION: In the present study results of Vitek and E-strip were almost comparable. In the advanced era of automation and computerized technology with manpower compromised labs, Vitek could be a better option for vancomycin MIC.

KEYWORDS : Methiciline Resistant Staphylococcus aureus (MRSA), Vancomycin (VAN), Vitek 2, E-strip Test, Cefoxitin (CX).

INTRODUCTION

Staphylococcus aureus (S.aureus) has been an important cause of human disease for more than 100 years. [1] S. aureus is responsible for many infections but , they are normal colonizers of various skin and mucous surfaces in humans as well. [1,3] S. aureus has been documented in a variety of infections ranging from minor skin infections & chronic bone infections to urinary tract infections and severe septicaemias. [1] S. aureus is one such bacterium which has been constantly evolving over time with regards to acquisition of complicated mechanisms of antimicrobial resistance and changing disease profiles. [1] The significant events in the evolution of antimicrobial resistance in S.aureus has been the development of methicillin resistance which has become a grave problem in many hospitals around the world.

Methicillin Resistant *Staphylococcus aureus* (MRSA) continues to increase, because of universal resistance of MRSA to β-lactams and effective alternative, vancomycin became the mainstay of treatment for serious infection. [1,2] Infections caused by isolates with an MIC considered susceptible according to the CLSI, with susceptibility defined as an MIC ≤ 2mcg/ ml. [1,3] Vancomycin sensitive MRSA isolates with indicated VAN MIC of 2mcg/ml may still result in treatment failure. [1] The rising MICs of Vancomycin among Vancomycin susceptible S.aureus (VSSA), referred to as the " Vancomycin MIC Creep", has caused a re-evaluation of vancomycin susceptibility criteria in cases of complicated infections like bacteraemia and or pneumonia. [1]

As less number of studies are available in view of increasing MICs of vancomycin in MRSA isolates and variations in the MICs according to the method employed, the present study was proposed to assess the reduced susceptibility of vancomycin in MRSA isolates in an Indian tertiary care facility and comparison of two methodologies that is Vitek 2 & E-Test methods. [1]

MATERIALAND METHODS

A total of 10680 various specimens were received and processed in the Clinical Microbiology laboratory, Tertiary Care Hospital over a period of 6 months (July to December 2020) out of which 210 S. aureus were included in this observational study. S. aureus were identified by gram staining, catalase, slide and tube coagulase test, etc as per the standard protocol.

Screening for methicillin resistance was done by cefoxitin 30µg disc as per CLSI guidelines. A total of 76 MRSAs obtained from different clinical samples. Vancomycin susceptibility testing was performed on these MRSA isolates by two methods viz. Vitek 2 & E-Test.

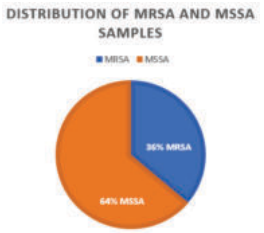
E-Test: The E-Test strips were brought to room temperature. The inoculum was prepared by making a direct broth suspension of isolated colonies selected from an overnight growth on blood agar plate. The suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland. Lawn culture from this suspension was made on Muller Hinton Agar using a swab according to standard protocol. E-Test strips (HiMedia) for vancomycin (0.016 to 256 µg/ml) were applied on the plates after being dried for 10 minutes and the plates were incubated at 35.0°C for 18-24 hours. MIC was measured where a clear defined zone of inhibition intersected the strip .

Vitek 2: A pair of plastic tubes was used for each isolate. Three mililitre of 0.45% NS (normal saline) was taken in each tube. Colonies from an overnight growth were picked up with an inoculating wire and emulsified in the first tube. The turbidity equivalent to 0.5 Mc Farland was measured using densicheck. A 280µl ml from first tube was pipetted into the second tube. Then GP ID and P628 cards were put in the first and second tube respectively. The cards with the test tubes was fed into the Vitek 2 machine for identification and antibiotic sensitivity testing where bacterial suspension got vacuum filled in both the cards. The cards were then inserted in the incubator-reader of the Vitek 2 system and the results were expressed as MIC values in µg/mL. (Vitek 2 Compact Systems Version: 06.01).

RESULTS

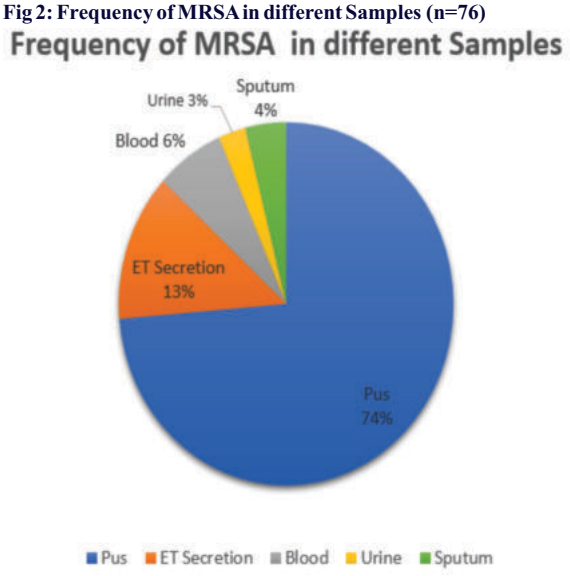
A total of 10680 various specimens like Pus, Blood, Urine , Body fluids etc ,were received and processed in the Clinical Microbiology laboratory. Out these a total of 210 samples were S. aureus amongst which 76 were MRSAs on which Vancomycin susceptibility testing was performed by two methods that are, Vitek 2 & E-Test.

Fig 1: Distribution of MRSA and MSSA Samples



A total of 76 MRSA isolates were obtained from different clinical samples of patients attending various OPDs and IPDs of the hospital. Pus was the predominant sample followed by endotracheal secretions, blood, and sputum. The distribution of MRSA isolates in relation to various samples is provided in [Fig-2].

Fig 2: Frequency of MRSA in different Samples (n=76)



On further analysis we found that vancomycin MICs of MRSA isolates were measured by E- strip Test & Vitek 2 method (MIC measured in µg/ml) . All the strains of MRSA found susceptible to vancomycin (MIC≤ 2µg/ ml) by both the methods. MIC of Vancomycin by E- strip test was approximately in the range of 0.75 –2µg/ml. Maximum specimens by both the methods had MICs of 1.0 µg/ ml. Also, we found that some specimens have reached MICs of 2 µg/ ml. (Table1)

Table 1: Comparison of Vancomycin MICs determined by Vitek 2 and E-test (n= 76)

Methods	MICs						
	≤0.5	0.75	1.0	1.5	2.0	≥2.0	Total
Vitek-2	--	08 (10.5 %)	44 (57.8 %)	13 (17.1 %)	11 (14.4 %)	-	76 (100 %)
E-strip	--	11 (14.4%)	40 (52.6%)	13 (17.1 %)	12 (15.7 %)	-	76 (100 %)

Sensitivity to relevant antibiotics for the above isolates done through Vitek 2 automated system. Of the 76 MRSA isolates, all the 76(100%) isolates were sensitive to Vancomycin and Linezolid, 49(64.4%) to Doxycycline, 43(56.5%) to Gentamycin, 39(51.3%) to Clindamycin, 26(34.2%) to Erythromycin, 25(32.8%) to Ciprofloxacin. All the strains of MRSA found resistant to Penicillin and Cefoxitin in Vitek 2 automated system.

Sensitivity to relevant antibiotics for the above isolates done through Vitek 2 automated system. Of the 134 MSSA isolates, 134(100%) isolates were sensitive to Cefoxitin, Vancomycin and Linezolid, 110(82.0%) to Ciprofloxacin, 109(81.3%) to Doxycycline and Amikacin, 103(76.8%) to Clindamycin, 93(69.4%) to Erythromycin, 60(44.7%) to Penicillin-G.

Table 2: Antibiotic susceptibility of the Staphylococci

Antibiotics	MRSA (n=76)	MSSA (n=134)
Penicillin-G (P)	00 (0%)	60 (44.7%)
Erythromycin (E)	26 (34.2%)	93 (69.4%)
Clindamycin (CD)	39 (51.3%)	103 (76.8%)
Cefoxitin (CX)	00 (0%)	134 (100%)
Doxycycline (DOX)	49 (64.4%)	109 (81.3%)
Ciprofloxacin (CIP)	25 (32.8%)	110 (82.0%)
Linezolid (LZ)	76 (100%)	134 (100%)
Vancomycin (VAN)	76 (100%)	134 (100%)
Gentamycin (GEN)	43 (56.5%)	106 (79.1%)
Amikacin (AK)	45 (59.2%)	109 (81.3%)

Fig:3 Showing VAN E-strip method

DISCUSSION

In the recent years, *Staphylococcus aureus*, more so MRSA is becoming a global challenge causing wide range of infections both at the community and hospital setup.[6] The overall prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) is alarmingly high and making the vancomycin (VAN) a mainstay of therapy for life threatening MRSA infections. Indiscriminate usage of vancomycin has led to the emergence and spread of resistance to the same amongst the MRSA isolates is a matter to worry. Minimum Inhibitory concentration (MIC) is the right indicator while selecting a proper antibiotic for treating serious infections.[6]

In present study, about 210 S.aureus strains were obtained from different clinical samples like pus, blood, urine, endotracheal aspirates. Of these, 76 (36.1%)were MRSAs which is marginally same as mentioned by Raghabendra adhikari et al. (35.5%).[7] Reema et al.(46%) and Mita D et al.(47.5%)found slight increase percentage of MRSAs.[13,15] Nasiru abdullahi et al. ,and Kumari N et al. reported only 26% MRSA isolates in their studies.[10,11] This variability in percentage of MRSAs may be due to difference in antibiogram pattern of MRSA in different geographical areas. Therefore, the choice of antibiotic for the treatment of infections caused by MRSA should be guided by the antibiotic susceptibility test of the isolate and the current antibiotic policy. [6]

As shown in Fig 2, Pus was the predominant sample followed by endotracheal secretions, blood and sputum for the MRSA isolation. *Staphylococcus aureus* is well known cause of various infections, most commonly causing abscesses, skin and soft tissue infection. [6]

In the current study we compared the vancomycin MICs of MRSA isolates by E-Test & Vitek 2 (MIC in µg/ml) method (Fig: 3). All the MRSA isolates were susceptible to vancomycin (MIC≤2µg/ mL) by both the methods. Vancomycin MIC detected by both the methods in the present study are almost similar with slight variation. About 14.4% isolates showed MIC of 0.75µg/ml, 52.6% with 1µg/ml and 15.7% strains showed MIC value of 2µg/ml by E- strip Test. Whereas with Vitek 2 method, 10.5% revealed MIC value of 0.75µg/ml, 57.8% strains with 1µg/ml & 14.4 % showing 2µg/ml of MIC. Similar results were seen in study by Anitha T.K. et.al. [6] On the contarary Robin et al., reported Vancomycin MIC values of 8-16mg/l by MRSA strains in their trial which were found to be resistant to Vancomycin. [16] Diaz et al.4 also reported no significant differences between E-Test method & BMD for vancomycin MIC detection for MRSA isolates.[8] In a study done by Himani et al, E-test corelated better with BMD method than Vitek 2 preferring E-Test method for determining vancomycin MICs than Vitek 2.[1] Anitha T.K et al. also reported no significant differences between E-Test method & BMD (Broth microdilution) for vancomycin MIC detection for MRSA isolates.[6]

The limitation of our study is inability to perform BMD which is a gold std. BMD is very laborious, time consuming, needs expertise and intersubjective variations during interpretation. So in small laboratory setup with limited resources, it is difficult to perform on routine basis. Hence vitek and E-strip both are very compatible methods with respect to BMD.

In the antibiotic susceptibility report of Vitek 2 automated system for the MRSA strains in the present study, maximum sensitivity was seen with Linezolid and Vancomycin (100%). In our study all the strains of MRSA found susceptible to vancomycin (MIC≤ 2µg/ ml) by both the methods. MIC of Vancomycin by E- strip test was in the range of 0.5 –2µg/ml which is similar to and very well correlated to studies conducted by Ranjan et al. and Mouton et al.[18,19]

Followed by Linezolid and Vancomycin, MRSAs showed maximal sensitivity to Doxycycline (64.4%) and Gentamicin(56.5%) . Large number of MRSAs showed resistance to Ciprofloxacin and Erythromycin. Variable rates of susceptibility to the same antibiotics were reported by Kumari et al. and Trivedi et al.[11,17] It may be probably due to the random usage of these drugs for empirical therapy.

CONCLUSION

Vancomycin has till now remained the cornerstone of treating serious MRSA infections. Reduced susceptibility to vancomycin in MRSA isolates has now therefore become an area of concern and research. MRSA isolates with higher MICs, even within the susceptibility range, are being observed more frequently which results in treatment failures with vancomycin. Because of the discrepancy that exists in vancomycin MIC results from different methods, the prediction of outcome of serious S.aureus infections should be taken into account. In the present study results of Vitek and E-strip were almost comparable. In the advanced era of automation and computerized technology with manpower compromised labs, Vitek could be a better option for vancomycin MIC.

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